LYMPHOID TISSUE
AND RESISTANCE TO INFECTION

Joanne Vardakis

BOSTON UNIVERSITY

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LYMPHOID TISSUE AND RESISTANCE TO INFECTION

By

JOANNE VARDAKIS

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APPROVED BY

First Reader Genevieve Young
Assistant Professor of Biology

Second Reader Brenton R. Kurtz
Professor of Biology

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INTRODUCTION

Infection is known to occur when micro-organisms invade healthy tissues, passing the barriers normally present in the skin and mucous membranes, and proliferating in the living tissues. The infected host has natural defensive forces which are ready to destroy the micro-organisms, and, in a great many cases, they are sufficient to protect the body against infection. However, if the natural defensive mechanisms are overcome, the body cells are still capable of resisting the organisms. The products of infection serve as a stimulus to the body cells, thereby causing renewed cellular activity which results in the production of various specific defensive weapons - the antibodies.

There are thought today to be four ways (Boyd 1945) by which an increased resistance to infection in the animal occurs: by 1) an increase in the non-specific factors of resistance, such as a decrease in skin or mucosal permeability; 2) an alteration of tissues wherein they become intrinsically resistant; 3) an aggregation of phagocytes made available to combat the invading organisms; and 4) a secretion of antibodies into the blood and tissue fluids. These, all or in part, exert a continuous resistance against the invading pathogenic agents in order to rid the body of them and to neutralize their effects. This effective resistance of the host against infection is called immunity.

Historical

The nature of the specific defensive mechanism has long been a subject of extensive experimentation and investigation. Prior to 1883, there had been many theories as to what actually occurred. Pasteur (1876)

thought that micro-organisms were forced to retard their activity because of lack of nourishment, for he thought they used up some substance essential to their own growth and existence, and thus were unable to exist in that environment. This was called the "exhaustion theory".

Later there arose the "retentive theory" (Kolmer 1925) of Chauveau, which is just the opposite of the "exhaustion theory". He considered it more probable that metabolic products from the bacteria accumulated in the blood, and these products had an inhibitory effect on the organisms.

The preceding, however, are at present obsolete theories, and therefore of historic interest only. Since then, through a series of experiments in which he injected dyes and bacteria into the body and observed the part certain body cells played in both ingesting and digesting these foreign agents and thus overcoming infection, Metchnikoff (1883) evolved a new theory on the problem of immunity and the mechanism of cure. This was followed by investigations showing the importance of body fluids, and, in turn, led to subsequent experimentation and to supplementary evidence as to what occurred during immunity. The following two theories include the most important findings (Kolmer 1925): 1) the "cellular theory" which ascribes protection and curing of infections to the activity of the cellular elements of the blood, especially the leucocytes, and 2) the "humoral theory" which attributes the power to resist infection to the body fluids. The former is congruous with Metchnikoff's "theory of phagocytosis" (1883), whereas the latter is similar to Ehrlich's "side-chain theory"(1897).

Metchnikoff's early work had been primarily with the nutrition of amoebae and other protozoa, how they engulfed food by means of pseudopodia, and the fate of the ingested material. He became interested in leucocytes and their ability to surround and engulf foreign matter

because of Cohnheim's description of inflammation. He tried to correlate the two separate functions by studying infections from the lowest animals up to man, and found that leucocytes possessed the same property as the amoebae in that they could both ingest and digest bacteria. In his own words "the intracellular digestion of unicellular organisms and of many invertebrates had been hereditarily transmitted to the higher animals, and retained in them by the ameboid cells of mesodermic origin. These cells, being capable of ingesting and digesting all kinds of histologic elements, may apply the same power to the destruction of micro-organisms" (Kolmer 1925). He named the cells capable of acting as scavengers, phagocytes.

His early cellular theory merely stated that phagocytic cells existed, which were capable of digesting bacteria, both dead and alive, and also cellular debris. If the infection were greater than the leucocytes present could cope with, the host would not recover, and vice versa; i.e., if there were sufficient phagocytes present, the host could fight off the infection. This early theory did not consider the various types of phagocytes.

Metchnikoff later divided the phagocytes into microphages or polymorphonuclear leucocytes and the macrophages or large mononuclear cells which occur in the blood stream as mobile cells and fixed in tissue as sessile cells. The former are deemed of primary importance in bacterial infections, whereas the latter were thought to be only indirectly active. Kyes (1916), however, showed that they play a great part in the high immunity of pigeons against virulent pneumococci.

The "cellular theory" could not be defended against the "humoral theory" which ascribed the power to resist infection to the body fluids. Denys, Leclef, Flugge, Nuttal, Pfeiffer, and others (quoted from

Kolmer 1925) demonstrated that bacteria could be destroyed and recovery from infection could occur without the influence of phagocytosis. Von Fodor (1887) was the first to contribute to this theory. He discovered that rabbit's blood killed anthrax bacilli in vitro independent of phagocytes. This was confirmed by Nuttal (1888) and Pfeiffer (1894). Ehrlich (1897) presented his "side-chain theory", which is purely theoretical and offers an explanation for the observations of the early contributors to the "humoral theory".

It was first presented in 1885 to explain the processes of nutrition. The cell, according to Ehrlich, had two functions. The first was physiologic in nature, as, for example, the transport of oxygen by erythrocyte or the reception of stimuli by sensory cells. The second was nutritional and dealt with the metabolic activities of the cell. The latter function was the one that Ehrlich was concerned with in his hypothesis on immunity. Essentially he thought the cell consisted of two parts, a nucleus which determined the nature and property of the cell, and a large number of side chains, or receptors, which entered into chemical combination with food particles in the circulation. Through an adsorptive mechanism, the substances became incorporated into the molecule. Thus, in an infection, the receptors picked up the toxic substances which were distributed in the blood, by means of suitable side chains.

According to Ehrlich then, antibodies were free receptors which had become very abundant due to the presence of a toxin, and had consequently broken away from the cell and floated around in the body fluid. They united with the toxin to counteract its effects on the body. In his experiments, he repeatedly injected a horse with diphtheria toxin which resulted in the stimulation of the cells by the toxin to produce

a larger number of receptors (antibodies). An injection of a serum saturated with receptors against the diphtheria toxin could counteract its effects as the receptors would unite with the toxin and protect the animal against infection.

In brief then, Ehrlich's theory states that a toxin (antigen) must be strong enough to stimulate the production of more receptors than are normally present, and must enter into chemical combination with the receptors (antibodies). It is, however, unlikely that a large number of various receptors are present at all times in the body as Ehrlich has stated.

Both Metchnikoff's and Ehrlich's theories, the former being biological and the latter biochemical, are incapable of explaining the exact entire mechanism of immunity, but each contributed enough evidence to stimulate further research in this line, in the fields of biology, biochemistry, and biophysics. They have made possible the explanation of many phenomena concerning pathogenic agents and their effects on the invaded hosts.

Antibody Production and Phagocytosis in Infection

Metchnikoff modified his theory, after the evidence presented for the presence of antibodies to counteract infection, maintaining that these substances were products of the phagocytes. It cannot be definitely stated that immunity occurs by the action of phagocytes or antibodies alone. There is an interplay of both mechanisms as shown by Denys and Leclef (1895). They showed that bacteria were more readily destroyed by leucocytes when immune serum was added to the infected host. These antibodies which facilitated phagocytosis of bacteria are called "opsonins".(Wright and Douglas 1903). There are thought to be two components of opsonins: an antibody which is thermostable, and

another substance which is thermolabile which appears to be similar to the complement. They are both present in normal serum, but the antibody concentration is greater in immune serum. Both components must be present in immune serum, however, to effect maximum phagocytosis.

Many theories have been introduced since Ehrlich's "side-chain theory" to explain the mechanism by which antibodies and antigens are combined, and thereby neutralized. Bordet used adsorption and colloidal chemistry to explain this phenomenon. He believed that the combination of a toxin and its antitoxin was similar to that of starch and iodine; i.e., an adsorption between colloids of opposite electric charges. If a large amount of antitoxin were present, the toxin would absorb sufficient antitoxin to destroy its toxic properties. Many other theories such as this one have been presented, but each is purely hypothetical, although quite reasonable.

The Site of Antibody Formation

One of the most important questions of the problem of immunity is the site of antibody formation. From what source do antibodies arise? The reticulo-endothelial system has been shown by several investigators to be the site of formation of antibodies. It is quite plausible that they are produced in this tissue as it is widespread throughout the body and plays an important part in phagocytosis. To make more exact studies, the investigators have studied antibody formation after the extirpation of various organs, and after injury to the reticulo-endothelial system. They have also studied the antibody content of various organs in an immunized animal, and they have attempted to stimulate the production of antibodies in tissue cultures. The results of these various studies will be presented in a later section.

The latest investigations seem to ascribe the source of antibodies

to the lymphocyte (Dougherty, Chase, and White 1944). Metchnikoff suspected that this cell was instrumental in producing antibodies, but he was unable to present any conclusive evidence. Recent investigations have shown quite conclusively that the lymphocyte plays a major role in antibody production. Polymorphonuclear leucocytes and macrophages as well as lymphocytes may be necessary, the last, however, being more important. Evidence for this theory will also be presented in a later section.

Lymphoid Tissue and Its Function

Lymphoid tissue although not too abundant is scattered throughout the entire body. It differs from the reticulo-endothelial tissues in that it consists of a preponderance of lymphoid elements, lymphocytes, rather than macrophages. They are held together in a supporting framework of reticular cells, fibrous and elastic tissue, and sometimes muscle fibers (Drinker and Yoffey 1941). It is estimated that it comprises 1 per cent of the body weight and that it is found in lymph nodes or glands, in the mucous membranes, especially of the alimentary canal, in the spleen, and the thymus.

A typical lymph node drains lymph. The lymph enters the node through afferent lymphatics that converge at the periphery and leaves it through efferent lymphatics which emerge near the hilum of the gland. As the lymph passes through, it tranverses a meshwork of sinusoids lined by phagocytic cells and divided by a reticulum which serves to bar the passage of foreign particles, as well as to phagocytize. The primary function of lymphoid tissue is exercised principally during a bacterial infection, in that it serves as a filter of bacteria. However, since a state of "subinfection" exists even in health, the lymph glands continuously filter out the bacteria which have entered

the lymph through minute "physiological defects" in the skin and mucous membranes (Adami 1910).

Reticulo-Endothelial System and Phagocytosis

The reticulo-endothelial system also removes bacteria and foreign particles from the blood and lymph. Some evidence for this was found when a number of colloidal dyes and suspensions were injected intravenously into an animal and were later found deposited in the liver and bone marrow as well as in the spleen and lymph nodes, rather than being eliminated in the bile or urine (Capell 1929-1930). We have seen how the formation of antibodies is also necessary to counteract infection. Do the macrophages or lymphocytes give rise to them? Is the reticulo-endothelial tissue more instrumental in producing antibodies or is the lymphoid tissue of primary importance? To answer these questions it is first necessary to define an antibody.

The Nature of Antibodies

Antibodies are actual chemical substances (modified globulins) (Boyd 1945) and are now inseparably associated with serum globulin. Since lymph contains varying amounts of globulin, it is natural to expect to find antibodies in lymph corresponding to the amount of proteins present which are identical with the serum globulins. The level of serum protein was shown to increase when adrenocorticotropic hormone (A.C.T.H.) and adrenal cortical hormones were administered. The initial effects of the adrenal cortical hormone and A.C.T.H. were seen in all of the lymphoid tissues. It caused a degeneration or "dissolution" of lymphocytes (White and Dougherty 1944). It is quite plausible then, that the lymphocyte "dissolution" caused the increase of serum protein level of the body.

Extracts of lymphoid tissue, which consist mostly of lymphocytes,

showed the presence of four protein components, one of which was gamma globulin. The demonstration of normal gamma globulin in the lymphocyte suggests that labeled globulins or antibodies might be present in lymphocytes of immunized animals (White, Dougherty, and Chase 1944).

I have briefly presented in the foregoing paragraphs the scope of this present thesis; i.e., lymphoid tissue, and especially the lymphocyte, and its role in antibody production. In the pages to follow, I shall present in much more detail the investigations and theories which have recently contributed to this new hypothesis on antibody production and its association with lymphoid tissue.

LYMPHOID TISSUE

Reticulum, or reticular tissue, is a primitive form of connective tissue which is thought to be the result of embryonic mesenchyme. The meshes of the network are relatively rich in cells, and when these cells are lymphocytes, the tissue is called lymphoid tissue. Lymphoid tissue may therefore be said to be composed of two types of tissue: 1) a fine fibrous connective-tissue framework, the reticular tissue, and 2) a mass of spherical cells which fill the interstitial spaces of the reticulum, the lymphocytes. It is found spread out throughout the body, in lymph nodes or glands, in the mucous membranes of several organs, in the spleen, and in the thymus (Piersol 1930).

Lymph Glands - Structure and Function

The lymph glands are of various sizes and generally kidney-shaped, the indented region known as the hilum (Schafer 1929). They are found mainly in connective tissue and are surrounded by a capsule of fibrous connective tissue which contains some elastic elements and which increases in abundance as the animal grows older. The capsule gives rise to branching and anastomosing trabeculae composed of the same tissue. The spaces between the trabeculae contain the reticulum or stroma of the gland, and superimposed upon it are the lymphocytes or the parenchyma of the gland. The latter is so arranged as to form a cortical region and a medullary area. The cortical region is found on the convex side of the gland, and it contains follicles or secondary nodes, the peripheries of which are dense zones, whereas the centers are more diffuse zones called the germinal or reaction centers (Maximow and Bloom 1946). The cells here are larger and of various sizes, showing oftentimes mitotic activity, as it is the center of proliferation of the cells of the gland. The medullary area is composed of irregularly arranged cords of

lymphoid tissue which have no germinal centers and which are originally formed in the cortex. Lymph sinuses separate the medullary cords. They are also found separating the cortical follicles from the trabeculae and from the capsule.

Afferent lymph vessels enter the gland at the convex side and, after branching in the capsule, run along into the channels or sinuses of the cortical and medullary areas towards the hilum, join there and leave the gland through efferent lymph vessels. As the lymph passes through the gland, it acquires a great number of lymph corpuscles from the cortical follicles. These are the lymphoblasts, which through kariokinetic division give rise to lymphocytes. (Schafer 1929).

The lymph glands possess a copious blood supply. Their main artery enters at the hilum, sends branches along the trabeculae, where they break up into capillaries in the lymph sinuses of the medulla and the cortical follicles. These then pass into venous vessels in the periphery of the cortex, cross the sinuses, enter the trabeculae, and go toward the hilum parallel to the arteries where they converge and emerge from the gland (Schafer 1929). The glands also contain both medullated and non-medullated nerves, although they are not too abundant. They supply the blood vessel walls, the capsule, and the trabeculae, but they have not been demonstrated in the cortical nodules or in the medullary cords (Weatherford 1946).

Follicles or nodules similar to those found in the cortex of the lymph glands can occur alone and are thus found widely distributed throughout the mucous membranes; or they may be aggregated as in the Peyer's patches of the terminal portion of the ileum, or in the lingual and pharyngeal tonsils (Sabotta 1903). They do not possess a capsule, medullary cords, or lymph sinuses, but they do have

germinal centers. They are connected with lymph vessels by irregularly formed lymph sinuses which surround them and are in close connection with the superficial epithelium of the body.

The lymph glands serve to filter lymph because of the phagocytic activity of the reticular cells (Maximow and Bloom 1946). Erythrophagocytosis also occurs here when there are hemorrhages in neighboring tissue. Foreign particles as those of coal dust and pathogenic bacteria are ingested and destroyed by the macrophages. Lymph glands are also instrumental in the production of lymphocytes. They are not necessarily produced in the germinal centers as proliferation of these cells occurs before birth when there are no such centers present (Drinker and Yoffey 1941). Active multiplication has also been observed in diffuse lymphoid tissue which is completely lacking in germinal centers.

The Spleen - Structure and Function

The spleen, found in the peritoneal cavity, is the largest mass of lymphoid tissue of the body, but it is more closely connected with the blood stream than with the lymph stream. It acts as a filter of blood especially during immune reactions, for it allows the blood to come in close contact with its macrophages due to another type of blood vessel, the sinusoid which connects the arteries and veins (Knisely 1936). It varies from the capillaries in that it does not have a constant diameter all along its length, and its walls are not formed by a continuous layer of endothelium, but by irregularly scattered phagocytic cells. The sinusoids have been shown to store vital dyes and to phagocytose bacteria, functions not demonstrated by the capillaries. Sinusoids do not possess a connective tissue layer in their walls but instead a dense, membranous network of reticular fibrils. As the circulation is sluggish through here, the sinusoid is a great help in purifying the blood, and

serves therefore as a very efficient filter of blood.

The spleen is surrounded by a thin elastic capsule, which is thickened at the hilum where it is attached to the peritoneum and where the arteries and veins enter and leave the organ, respectively. The capsule gives off the trabeculae, which branch and anastomose, penetrating the organ, and forming part of its network. The spaces between the trabeculae are filled with white pulp which is typical lymphoid tissue, and the red pulp which is a paste-like, dark red mass of cells and which does not resemble lymphoid tissue at all. The distribution of white pulp and the red pulp varies due to the arrangement of the blood vessels, the former being closely associated with the arteries, and the latter with the veins. The lymphoid tissue which accumulates around an artery forming a sheath is called the Malpighian body. Its stroma consists of a network of reticular fibers connected to primitive reticular cells and phagocytic reticular cells, and the parenchyma consists of free lymphocytes which are distributed to form diffuse and nodular lymphoid tissue. The amount of lymphoid tissue varies with the condition of the body; for example, during myeloid leukemia, it is practically absent, whereas in lymphatic leukemia, it predominates over the red pulp. Although the spleen is mainly a hemopoietic organ after birth, lymphocytes are also produced here in the white pulp. They migrate into the red pulp where they are picked up by the terminal veins and are thus brought into the circulation.

The Thymus - Structure and Function

Another relatively large mass of lymphoid tissue is the thymus, a broad, flat bilobed structure, the majority of which is located beneath the upper part of the sternum in the thoracic cavity, while an elongation extends on either side of neck (Weatherford 1946). The whole organ is surrounded by a capsule. The two lobes are macroscopically

divided into several lobules which microscopically are composed of two different areas, the cortex and the medulla, and these are bounded by connective tissue septa which are a continuation of the capsule. Through the examination of stained serial sections, it has been seen that the medullary areas of all the lobules are continuous (Maximow and Bloom 1946). There is then a medullary stalk which gives rise to all the medullary tissue. This is nearly completely surrounded by cortical tissue. The framework of the gland consists of a stellate reticulum, and within these meshes, there are small cells which resemble lymphocytes, the thymocytes. There are more thymocytes in the cortex of the lobules than in the medulla.

The cortex is similar to the cortical layer of the lymph glands. The cells of the reticulum are similar to those found in other lymphoid tissue; i.e., they possess large pale, oval nuclei and a stellate cytoplasm. They do not, however, exhibit the ability to ingest and digest foreign particles as shown by the cells of mesenchymal origin, and therefore are not considered part of the reticulo-endothelial system (Weatherford 1946). The thymocytes of the cortex possess a small, circular, darkly staining nucleus with only a fine layer of basophilic cytoplasm surrounding it. There are macrophages scattered throughout the reticulum of both the cortex and the medulla.

In the medulla, the thymocytes are not as dense as in the cortex, and consequently exhibit more reticular cells, and oftentimes leucocytes, especially eosinophile myelocytes and plasma cells (Weatherford 1946). There are no germ centers here, nor anywhere else in the thymus. This diffuse area possesses the most characteristic structures of the thymus, Hassal's or thymic corpuscles, spherical bodies which probably start as only one cell with an enlarged nucleus and a hyalinized cytoplasm. The

cytoplasm appears to enlarge in concentric layers, and eventually it obliterates the nucleus, incorporating, in the meantime, neighboring cells, compressing them, and thus making them part of the corpuscle. The central area may become calcified, or it may become vacuolated due to fat accumulation. The hyaline substance is considered similar to the colloid of the thyroid. These corpuscles are considered as degenerations of the gland; as a degeneration of involuting blood vessels (Jordan and Horsely 1927), or as the keratinization of the surface cells in the obliterated lumen of the precursor of the thymus, the third pharyngeal pouch (Kingsbury 1928).

The thymus has a copious blood supply as do all the other lymphoid structures. Arteries enter in the medullary stalk and are mainly found between the cortex and medulla with branches in both areas. Those branches in the cortex empty into veins which run along the septa between the lobules, whereas those of the medulla empty into veins which are found there. The medulla is more vascular than the cortex. There are not many lymph vessels present. The lymphatics which are present run along the interlobular connective tissue and empty into the mediastinal and tracheobranchial lymph nodes. There are no lymph sinuses present. Innervation consists mainly of sympathetic fibers and a few branches from the vagus nerve. They terminate along the blood vessels and are of importance in vasomotion. There are a few free endings in the medulla. The nature of the thymocytes is still a controversial matter. They are indistinguishable from the small lymphocytes (Hammar 1905, 1921), but whether they are real lymphocytes is debatable, for they are not arranged as in the other lymphoid structures with cords and germ centers. Both cells are morphologically and serologically identical, but some investigators believe that the thymocytes are

migratory lymphocytes, of mesenchymal origin, which invade the epithelium from without (Maximow 1912). Others believe that their origin is in entodermal reticulum (Winiwarter 1924). It has been seen, however, that the reticulum develops by the vacuolization of the epithelium and the thymocytes developed as further differentiation of these same cells (Stohr 1906). On the whole, however, the majority of the evidence points to the theory that they are identical.

The thymus is an organ which undergoes involution when the animal is still quite young. At birth, it is a relatively large organ, and weighs, in the human, between five and fifteen grams. It grows rapidly for two years but the cell proliferation slows down after that until puberty is reached. Involution begins during the period between two years and puberty, and is evidenced by a gradual diffusion of the cortical lymphoid elements. A compression of the epithelial reticulum and a gradual replacement of these areas by adipose tissue occurs. The medulla does not atrophy until after puberty. The thymus, even though it involutes, persists in adults and even in the aged. Atrophy of the thymus can be spontaneously produced by the repeated injection of sex hormones, by fasting, toxins, and morphine (Selye 1936). The reverse may also occur. Adrenalectomy results in the persistance of the thymus.

The function of the thymus is not too well known. It is known to produce lymphocytes, a few plasma cells, and myelocytes. It has been ascribed in endocrine function due to the fact that it is so large at birth and gradually atrophies. However, this has not been substantiated. Extracts of the thymus have been shown to contain a growth-promoting factor. (Hammar 1937). When these extracts were fed to tadpoles, they grew very rapidly. Autoclaving destroyed the growth-

promoting factor which Hammar believed was due to the presence of vitamin B in the thymus.

The Reticulo-endothelial System and its Function

Lymphoid tissue is intermingled with reticulo-endothelial elements. Individually, the reticulo-endothelial elements are: the macrophages of the loose connective tissue, the reticular cells of the lymphoid and myeloid tissues, the von Kupffer cells in the liver sinuses, the lining cells of the sinuses of the adrenal gland and hyphophysis, and the adventitial cells around the blood vessels (Schafer 1929). Collectively, they are said to comprise the reticulo-endothelial system. (Aschoff 1924). All these cells have the ability to take up particulate matter and to store foreign substances brought to them in colloidal solution. Macrophages, then, are those cells in the connective tissue or blood which selectively store colloidal vital dyes, which they are capable of accumulating from a very weak solution. The polymorphonuclear leukocytes are also phagocytic cells, but they do not take up vital stains as do the macrophages of the reticulo-endothelial system. These are the microphages of Metchnikoff.

The same mechanism which frees the body of particulate matter was also shown to be one which rapidly ingests and digests bacteria which have become incorporated into the body. This occurs especially in the spleen, liver, and bone marrow.

Earliest evidence for phagocytosis in the reticulo-endothelial system was found in the prebacteriological days when many investigators found that particles of vermilion and carmine, when intravenously injected, were not eliminated but persisted in the various organs. Metchnikoff, using a number of dyes and suspensions also demonstrated this same phenomenon. It was not until 1904 that Ribbert first showed

that the phagocytic cells had an affinity for dyes that were of a colloidal nature. This was further shown by Aschoff who collectively listed those cells which were found all over the body and had the capacity to take up these dyes into the reticulo-endothelial system. The cells are said to be vitally stained. These vitally-staining cells and no others also took up fine suspended particles of quartz and carbon. (Ribbert 1904). The injection of bacteria into normal animals in the same manner resulted in their active phagocytosis by the reticulo-endothelial cells, particularly those of the lungs, spleen, and bone marrow, and an increase in specific antibodies was detected in these organs, before any increase was detected in the blood of the animals. (Pfeiffer and Marx 1898).

Lymphoid Tissue and Its Biological Significance

Recently, the significance of the lymphoid tissue in particular, rather than the reticulo-endothelial system as a whole, has been investigated for its role in phagocytosis and especially antibody production, and, consequently, the experimenters have tried to evaluate the role of the lymphocyte. They have used many methods to determine the biological significance of these lymphoid masses. (Drinker and Yoffey 1941). The main ones were irradiation, hemorrhage, growth of animals in sterile surroundings, complete lymphatic blockade, and the removal of scattered lymphoid masses.

Irradiation by X-rays was found to damage surrounding tissues as well as to cause involution of lymphoid tissue, therefore the results of these studies are not very dependable. Taylor, Witherbee, and Murphy (1919), found that large doses of X-rays caused a diminuition of circulating lymphocytes, whereas small doses caused them to

increase, after a variable length of time. Mottram (1931), found that the lymphocytes of irradiated rats adhered to capillary walls, which probably accounted for their decrease in circulating blood.

Mottram and Russ (1921) found that the dose of X-rays given to the tissues did not matter very much for lymphopenia resulted in any case. After a small dose of X-rays, an initial lymphocytopenia occurred immediately and lasted for a few hours. It was followed by an acute lymphocytosis which persisted from one week to ten days. Murphy (1926) elaborated this and said that after a brief lymphopenia the lymphocytes almost doubled in two days, and this increase lasted for fourteen days. There was a great deal of mitosis going on in the lymphoid tissues, but it subsided and returned to normal on the tenth day. Latta and Ehlers (1931), on the other hand, stated that after a short exposure there was marked degeneration in lymphoid tissue with a large fall in the number of lymphocytes and the blood leucocytes, both of which were reduced to one-fifth or less of their number normally present. Prolonged irradiation caused an extreme decrease in lymphocytes, but the cortical zones of the various lymphoid structures were reduced to loose connective tissue. The majority of the experimental work seems to favor the opinion that X-rays damage hemopoietic organs, but that lymphoid tissue is most sensitive. (Shouse, Marion, and Whipple 1931).

The effects of hemorrhage which results in anemia of lymphoid tissue were studied by Sjöval (1936). He repeatedly bled forty-seven rabbits and withdrew approximately 1000 cc. of blood from each animal within ninety days. The mean total weight of the lymphoid tissue of the experimental group at the end of the experiment was 10.185 gm., whereas it was 12.283 gm. in the control animals. The erythrocyte count fell from five million to three million cells per cubic centimeter, and the

bone marrow showed great erythropoietic activity. If it were true that lymphocytes were being drawn to the bone marrow to give rise to red corpuscles and granulocytes as some investigators believe, there should have been a great demand for them, and consequently an increase in lymphoid tissue. However, this was not the case, for the lymphoid tissue had been diminished by the repeated bleedings. Simultaneously the blood lymphocytes fell to approximately two-thirds of the number normally present. This latter change may be of no great significance in that Wiseman (1931) has shown that "the total number of lymphocytes circulating at any given time is not necessarily an index to lymphoid activity".

The effect on lymphoid tissue of raising animals in completely sterile surroundings was studied by Glimstedt (1936). He succeeded in raising eight guinea pigs under bacteria-free conditions. The general condition of their health was poor, and the amount of lymphoid tissue present was only 25 per cent of that in the control. The lymphoid tissue was devoid of germ centers so that Glimstedt concluded that bacteria are necessary especially as a stimulus to lymphoid tissue growth and also to the growth of the body, but to a lesser degree in the latter case. Since the germ centers failed to develop in the absence of bacteria, he agrees with Hellman that they are "reaction centers". The results are not conclusive, however, as one-third of the experimental animals died of causes unknown.

Complete lymphatic blockade was produced in three dogs by Blalock et al. (1937) by: 1) blocking the lymphatics in the neck and chest; 2) destroying the cisterna; and 3) interfering with the drainage of the mesenteric lymphatics. The animals lost weight and their lymphocyte and eosinophile counts were nearly completely abolished. The results indicate that the blood lymphocytes enter the blood through the lymph stream,

for lymphatic blockade caused the lymphocytes to disappear completely. Therefore, practically no lymphocytes enter the blood without the intervention of the lymph stream, so that neither the bone marrow nor the spleen supplies many lymphocytes to the blood.

Extirpation of various elements of the lymphoid tissue, such as spleen, or thymus has been attempted by many investigators. It seems, however, that lymphoid tissue functions as a whole so that some sort of compensatory mechanism is set up on the part of the rest of the lymphoid tissue when extirpation occurs (Drinker and Yoffey 1941). Whitney (1928) after he reviewed all the literature on this matter was of the general opinion that there is little proof that this compensatory mechanism exists in lymphoid tissue.

Lymphoid Tissue and Resistance to Infection

Lymphoid tissue has been seen to play a very important part in the defense reactions of the body from 1860 when Virchow first described his theory that lymph nodes acted as barriers; for example, when lymph, filled with foreign particulate matter, passed through a lymph node, it passed out of the other end in a rather purified state. He also demonstrated his theory by telling of a soldier who had had his arm tattooed with cinnabar, a red sulfide of mercury. The dye was later found only around the ramifications of the cortex of the nodule in the vicinity of the tattooing and nowhere else. None of it had even penetrated the next layer of follicles even though the particles were quite small. The lymph nodes, then, serve as a barrier. As McMaster and Hudach (1935) wrote; "Pathogenic bacteria carried in the lymph stream are often arrested in the glands through which this stream passes, with the result that the infection travels no further". (Drinker and Yoffey 1931). Thus the barrier theory was spread to include the other lymphoid tissues, even though

in some cases it did not seem at all justifiable. For example, the tonsils are thought to be a protection to the digestive tract. Similarly, the appendix and Peyer's patches have been ascribed a protective function which was easily accepted in that no known function had been ascribed to these tissues. In these lymphoid tissues of the gastrointestinal tract, however, the reticulum of the lymph simuses which effects filtration in the lymph nodes is not well developed (Drinker and Yoffey 1941).

Recent investigators have extended the barrier theory to include the concepts that 1) lymphoid tissues are reaction centers where toxic material is being filtered in the circulation, and 2) lymph nodes and consequently lymphoid tissue is the site of antibody production (Drinker and Yoffey 1941). The latter will be treated in a later section.

Hellman (1921) ascribed to the germ centers the name "reaction centers" because injurious material is said to be filtered out of the circulation here. The macrophages also aided in this, so that the lymph nodes had a dual role: 1) they not only prevented foreign matter from entering the blood through the lymph, but also 2) if such material did enter the blood, it would be filtered out in the reaction centers.

Hellman and White (1930) repeatedly injected a number of rabbits intravenously with Bacillus paratyphosus-B and found that the spleen had become enlarged, the intestinal lymphoid tissue had decreased and the rest of the lymphoid tissue had remained unaltered. They concluded that there was an increased activity in lymphoid tissues and that the germ centers were the main site of reaction. This conclusion was based on the changes which occurred only in the spleen. Ehrich (1929b), however, corroborated this. He injected staphylococci subcutaneously; these were virulent enough to produce only abcesses. There was hyperplasia in the

regional lymph nodes, and moderate lymphocytosis of the blood. The germ centers, which up to this point had been regressive, appeared after lymphocytosis reached a peak, increased in number and size, and the lymphocyte level of the blood fell gradually to normal. He favors the idea that the germ centers represent a reaction to toxic substances rather than centers of lymphocyte proliferation. When he repeatedly injected staphylococci he got hyperplasia of all the lymphoid tissue, even the thymus. The latter hypertrophied for a while, but then began to atrophy, whereas the rest of the lymphoid tissue continued in a positive direction and lymphocytosis occurred in the blood. After ten days, however, the lymphocyte level returned to normal, even though the injections were being continued.

Lymphocytes - Structure and Development

Lymphocytes are not very specialized cells either morphologically or functionally. They have a clear cytoplasm with no evidence whatever of granules. The nucleus does not appear to possess any lobules, though it is indented here and there. Lymphocytes may be of three types (Maximow and Bloom 1942). 1) The small lymphocytes are in the majority and very rarely exhibit mitosis. 2) The medium sized lymphocytes are less in number but are distributed all over. The nucleus is clearer and contains less chromatin, and the cytoplasm is slightly more abundant than in the small lymphocytes. These cells divide by mitosis and are the main source of lymphocytes of the body. 3) The large lymphocytes are found distributed everywhere, but are more abundant in the nodules. They possess a few vacuoles in their cytoplasm and also a Golgi net around the centrosome. They also divide by mitosis.

Lymphocytes are motile. Detailed work has been done on this by Lewis (1931, 1933), McCutcheon (1924), and Abramson (1927). They move by means of a single pseudopodium, with the nucleus always at the forward

end of the motile cell. They are only slightly phagocytic, and only with supravital staining and appropriate stains have they been seen to take up some azurophile granules, and their vacuoles take on a red color (Weatherford 1946).

Lymphocytes develop for the most part from those pre-existing in lymphatic tissue. In some cases they have been seen to arise from primitive reticular cells of lymphoid tissue (Downey and Weidenreich 1912). This probably occurs, however, when there is a great demand for them and the pre-existing lymphocytes are unable to produce the amount necessary. It is only the microphages, or those cells which do not take up the vital dyes that are capable of giving rise to lymphocytes. In autoplastic transplants of rat lymph nodes Jaffe and Richter (1928) found that all the lymphocytes degenerated and the remaining stroma of reticular cells gave rise to new lymphocytes.

The Fate of Lymphocytes

What is the fate of these lymphocytes? Is it possible that they give rise to plasma cells, granulocytes, erythrocytes, and monocytes? Jordan and Morton (1937) believe that "a plasma cell is a modified lymphocyte". Miller (1931), on the other hand, states that "plasma cells are not derived from lymphocytes". As for the blood elements, Bloom (1937, 1938a, 1938c) has done a great deal of experimental work with tissue cultures and upholds the idea that lymphocytes give rise to monocytes, to erythrocytes, and to granulocytes, or that the latter are transition forms of the lymphocytes. However, there are many other investigators who do not agree with him.

Heineke (1905) believes that the lymphocyte merely disintegrates and disappears from the circulation. After he had irradiated some lymphoid tissue, as early as two hours later, the lymphocytes began to

degenerate. Within four hours the chromatin particles were quite abundant. Complete digestion of this debris was effected a little more than 24 hours after the irradiation had taken place.

Another theory of the fate of the lymphocytes is that they return through the lymph capillaries to the germ centers where they are ultimately destroyed (Heiberg 1922-23). Sjoeval (1936) believes that lymphocytes merely return to the lymph nodes through peripheral blood vessels and consequently through peripheral lymph vessels. However, this does not seem very likely as the peripheral lymph vessels contain only one-tenth of the total number in the circulating lymph. Still another idea is that they leave the blood through the gastrointestinal tract, but this needs further investigation (Bunting and Huston 1921).

The Protein Constituents of the Lymphocytes

It does not seem likely that the cytoplasm of the lymphocyte is abundant enough to be associated with any metabolic process or even serve to transport fats or proteins. What function does the lymphocyte serve then? White and Dougherty (1945) showed that lymphocytes from lymph nodes contained normal gamma-globulin. Previous to this they had observed the influence of pituitary adrenocorticotropic hormone on lymphoid masses and the lymphocyte. There was a marked loss in weight of the former and lymphopenia, which suggested to the authors an approach to the fate of the lymphocytes. There occurred what they termed a "dissolution" of the lymphocytes which should have resulted in a release of protein nitrogen for metabolic use. They observed by use of the Tiselius apparatus that this protein nitrogen increased the globulin fraction of the serum proteins. A marked increase in this fraction of the serum proteins was concomitant with lymphocyte "dissolution". The lymphoid tissue was then

the source of these serum globulins. Kass (1945) has also demonstrated the presence of normal serum gamma-globulin in human lymphocytes when the subject also exhibited lymphoid hyperplasia. Adrenalectomy, on the other hand, gave evidence of excessively lowered total protein content of the serum, provided that a marked hemoconcentration was prevented.

Dougherty and White (1944) made a more elaborate study of the protein constituents of the lymphocyte. They made an electrophoretic examination of the protein-containing extracts of washed, lysed lymphocytes from the rabbit lymphoid tissue and this revealed specifically the presence of a protein component with electrophoretic mobility identical with gamma-globulin of a normal rabbit. Further experiments showed that the anterior pituitary controlled the release of serum globulins from lymphoid tissue (Dougherty and White 1944). But how did the lymphocyte obtain these globulins, through absorption, adsorption, or during reproduction at which time they were incorporated into their cytoplasm? Ehrich et al. (1945) took normal lymphocytes and incubated them with antibody-containing lymph plasma. They also injected antibody-containing serum into lymph nodes of living animals, removed the lymph several hours later, and determined the antibodies in both the lymphocytes and lymph plasma. There were no cases of absorption, adsorption, or incorporation of antibodies (globulin) by the lymphocytes. The demonstration of normal gamma-globulin in the lymphocyte suggested that in an immunized animal, they may contain antibodies also. This will be discussed later.

ANTIBODY PRODUCTION

The Reticulo-Endothelial System and Antibody Production

The site of antibody production has been one of the foremost questions in the problem of immunity, and, as a result, there has been a great deal of experimentation and investigation. Even though Ehrlich's "side-chain" theory did not satisfactorily answer this question, it served as a stimulant to other investigations. Many of them seemed to agree that the antibodies probably came from the phagocytic cells of the reticulo-endothelial system. Pfeiffer and Marx (1898) found that when they immunized animals against cholera bacilli, antibodies were found in the spleen, bone marrow, lungs, and even in the lymph nodes. This evidence lent a great deal of support to the theory of production of antibodies by the phagocytic cells. Several methods have been used to study the role of reticulo-endothelial system in antibody production. They are: 1) extirpation or damage to various organs of this system; 2) blockade; 3) the examination of the antibody content of various organs in immunized animals; and 4) the attempt to stimulate the production of antibodies in tissue cultures.

Hektoen and Carlson (1910) bled dogs from the carotid artery during the latent period of immunization after the injection of rat and goat corpuscles. During this period, no rise in antibody titre had occurred whatever. They then restored the blood with a transfusion from a normal animal and immediately got a marked production of antibodies, thereby indicating that the antigen had been rapidly removed from the blood. They also gave a normal animal which had been bled dry a transfusion from an animal in the latent period of immunization and found that it exhibited no rise in antibody titre, again showing that the antigen had been removed from the blood. What had happened to the antigen? Could it have

been stored in one of the body organs?

Extirpation and Damage of Organs of the Reticulo-Endothelial System

Extirpation has not been a very successful method to approach this study, as some simultaneous damage always takes place which might be capable of upsetting the entire metabolism of the animal (Boyd 1945). Deutsch (1899) found that if splenectomy took place immediately before the injection of the typhoid bacillus, there were no affects on antibody production; on the other hand, however, if splenectomy occurred three to five days after the injection, there was a marked decrease in the concentration of antibodies. He also observed that if the spleen of a recently immunized animal was prepared and injected into a normal animal, the latter exhibited the presence of antityphoid agglutinins, but at a very low titre. This was further studied by Luckhardt and Becht (1911), who injected rat and goat corpuscles intravenously into dogs, and shortly thereafter removed the spleen, minced it, and injected it into a normal animal. The latter showed a greater rise in antibody titre and at a much more rapid rate than did the animals that had been originally immunized.

Up until then, it was not known whether the cells of the spleen merely absorbed and retained the antigen and afterwards freed it so as to form a stimulus to other antibody-forming cells, or whether they themselves formed the antibodies. Topley (1930) clarified this somewhat with his experiments, which were more compatible with the latter idea. He intravenously injected paratyphoid bacilli into animals, killed them after intervals of 24 hours to 216 days, emulsified their spleens, and injected this tissue into normal animals. These exhibited agglutinins as early as 24 hours after infection, whereas the dead immunized animals showed no antibody titre whatever. This same reaction occurred if the

interval between immunization and removal of the spleen was longer. In animals where the interval was from 21-62 days, Topley injected a minimal dose of the same bacilli with the splenic tissues, but they did not exhibit a rapid secondary response, which should have occurred had they been injected with bacilli in the first place. From this, he believed that the splenic cells produced the antibodies rather than storing the antigen.

Other evidence that lends support to the reticulo-endothelial theory is that leukemic patients who apparently have severe damage of the reticulo-endothelial system possess an inferior antibody-producing power (Howell 1928). Destruction of macrophages by X-rays (Benjamin and Sluka 1908), by benzene (Rusk 1914), and by mustard gas (Hektoen and Corper 1921) all resulted in the view that any process which severely injured the cells of the reticulo-endothelial system simultaneously inhibited to a great extent, and oftentimes completely, the formation of antibodies. Removal of other organs such as the thyroid, stomach, and intestine, which are not parts of the reticulo-endothelial system, have revealed negative results.

Blockade

Blockade of the reticulo-endothelial system by intravenous injections of large amounts of India ink or colloidal iron so that much of this system is rendered inactive and unable to deal effectively with subsequent injections of antigenic material have resulted in divergent opinions. Standenath (1923) showed that it had a stimulating effect on antibody production, whereas other investigators seemed to agree that it is inhibitory (Gay and Clark 1924). However, these divergent results seem quite reasonable in that there is no way of knowing whether or not

complete blockade has taken place in the system. The results, therefore, are not conclusive.

Tissue Cultures

Carrel and Ingebrigsten (1912) added small amounts of goat erythrocytes to fragments of bone marrow and spleen, and they directly observed the presence of hemopsonins in the culture on the third day by the degree of phagocytosis present. On the fourth day hemolysins occurred which could be specifically absorbed from the fluid. Ludke (1912) was unable to demonstrate any positive results when he added killed cultures of typhoid bacilli directly to live tissue cultures. He was, however, able to remove fragments of spleen and bone marrow and cultivate it in homologous plasma from animals that had received killed cultures of typhoid bacilli, and he detected lysins and agglutinins in the culture fluid after two days. Beard and Rous (1938) were unable to demonstrate the formation of hemolysins or hemagglutinins when they introduced vaccinia virus into cultures of Kupffer cells, in vitro. The vaccinia increased in quantity and survived in the immediate association with these cells. No antiviral principle was elaborated by the Kupffer cells under such conditions. Other work along this line has not been too successful and the results are not at all conclusive.

Antibody Content of Various Organs of Immunized Animal

Haurowitz and Breinl (1932) used this method. They determined the arsenic content of various organs at intervals after the injection of an antigen prepared from arsanilic acid. They found that the liver and bone marrow which are mainly composed of reticulo-endothelial elements, contained the most antigen and retained it for the longest period of time.

They applied the theory that wherever the antigen is located the antibody is also there, in the process of being produced.

Rous and Beard (1934) found it was possible to loosen Kupffer cells from the liver of an animal by perfusion, after they had taken up particulate matter so that they could be more easily investigated. They used ferromagnetic iron oxide which when phagocytosed could be selected with a magnet, and, in that way, they were able to measure the amount of antigen absorbed, and consequently, how much antibody was present.

Mudd (1932), and Breinl and Haurowitz (1930), presented theories on antibodies, assuming that they were globulins, and that they were formed in the reticulo-endothelial system. Downey and Weidenreich (1912) had reported previously a shedding of cytoplasm by lymphocytes, with no apparent change in the cell. The material was not platelets because it contained no granules, and it eventually disintegrated in the thoracic duct. Sabin (1939) supplemented the work of these investigators and thereby presented the most conclusive evidence that antibodies were formed by the reticulo-endothelial cells. She used a marked antigen, a red dye, which could be identified in the phagocytic cells by its color. She observed that the material was placed in digestive vacuoles and the first signs of alteration occurred after the removal of the dye from the cell. The solid particles disappeared, so she concluded that it passed into the cytoplasm in a soluble form. When the dye could no longer be seen, she observed that there were antibodies in the serum, and, concomitant with this, there was a marked shedding of the surface film (ectoplasm) of macrophages with no damage to the cells themselves. From these observations she presented a theory for the mechanism of antibody formation: Antigens which are phagocytosed by cells of the liver, spleen, and lymph nodes cause an increase in the synthesis of globulins, and modify these

globulins into antibody globulin. Some of the cytoplasm of the phagocytosing cell is then shed, and there is an increase of globulin and antibodies in the plasma which can be shown by immunological reactions. This is by far the most conclusive work in the role of the reticulo-endothelial cells in the production of antibodies. It was just about this time, however, that McMaster and Hudack (1935) first presented evidence that lymph nodes might give rise to antibodies.

Role of Lymphoid Tissue in Antibody Production

Pfeiffer and Marx (1898) originally suggested that antibodies were produced in the lymph nodes which drain an area of infection, as well as in the spleen, bone marrow, and lungs, when they injected cholera bacilli into rabbits and guinea pigs. McMaster and Hudack (1935) in experimenting with this idea, came to similar conclusions. They injected two different antigens into mice, one in each ear. The corresponding antibody appeared first in the lymph node of the same side into which it was injected. This experiment shed no light on the site of antibody formation elsewhere in the body, or the exact cell which might give rise to them. It merely showed that agglutinins could be produced in the lymph nodes, and these results were compatable with both the reticulo-endothelial theory and the lymphoid tissue theory of antibody production. McMaster and Kidd (1937) further supplemented this work when they demonstrated that lymph glands are active in the formation of a neutralizing principle for vaccinia, possibly identical with that which appeared in the blood of animals that had been infected with the virus. The nature of the cell concerned was still questionable. Burnet and Lush (1941) using influenza and herpes virus, added to these experiments by suggesting that under similar conditions, antibodies could be found in the lymph nodes one or

two days before they were detected in the blood serum.

This experiment was followed by those of Ehrich and Harris (1942) in which they observed the cellular changes which occurred in a lymph node along with the production of antibodies. They also analyzed the composition of lymph in afferent and efferent lymph vessels. They used the popliteal lymph node of the rabbit's hind foot because it is the only one which drains that limb, and it is large enough to separate all the parts of system in evaluating the experiment. They injected typhoid vaccine into the hind foot of rabbits, and, at various intervals after the injection, collected lymph from the afferent and efferent lymph vessels of the node. Antibodies were present in the efferent lymphatic vein in two days, and reached the maximum level in six days. In all cases, the antibody titre was higher in the efferent lymph, and in some cases, the concentration was about 100 times that in the afferent lymph. There was an increase in the lymphocyte output in the efferent lymph before and during the rise in the amount of antibodies. The lymph nodes, through histologic studies, showed increased activity with an abundance of mitotic lymphoblasts. The node gradually filled with diffuse lymphoid tissue. Since the reticulo-endothelial cells remained quite inconspicuous during all this activity in the lymph node, it seemed evident that the lymphocytes were the chief cells concerned, and that they were an important factor in antibody formation. How the lymphocyte was concerned, they did not know. Ehrich and Harris suggested that since lymphocytes are unable to phagocytose, they probably absorb antigens or their split products, or act only after the antigen has been properly prepared by the action of phagocytes or some other mechanism. The exact action was unknown to them in any case. All they had shown was that there had been a lymphocytic response preceding and simultaneously with the formation

of antibodies.

The Lymphocyte and Antibody Formation

Following this experiment, Harris, Ehrich, Grimm, and Mertens (1945) worked on a still more limited area. They collected the efferent lymph from the popliteal lymph nodes after the injection of typhoid antigen, separated the lymphocytes from the lymph plasma, and compared the antibody titres in the lymphocyte extracts with those of the lymph plasma. In most instances, the antibody titre of the cell extract was substantially and consistently higher than that of the surrounding fluid. This experiment offered evidence that antibodies escaped from the lymphocytes into the surrounding medium. This led the authors to conclude that the lymphocytes were instrumental in the formation of antibodies.

At about the same time, Dougherty, Chase, and White (1944) published a report in which they stated that agglutinin and hemolysin titres had been obtained from extracts of washed cells secured from selected lymphoid tissue; i.e., lymph nodes and thymi of mice immunized against sheep erythrocytes. Although similar extracts of salivary glands and muscles of the same immunized animal were made, they were negative in all dilutions, even though they were higher in nitrogen content. Per unit of extractable nitrogen, lymphoid tissue had significantly higher agglutinin and hemolysin titres than did the sera of the same animals. They too concluded from their experiments that antibodies were concentrated in the lymphocytes.

Although previous experiments had shown that lymphocytes were the site of antibody production, this did not rule out the possibility that other cells also present in the infected area might be of some use in antibody production. Ehrich, Harris, and Mertens (1946) conclusively

proved that antibodies were absent from the macrophages during maximum antibody production. They injected dysentery antigen, both in saline and in saline and paraffin oil suspensions, into the hind foot of a rabbit. Although there was a considerable amount of antibody in the popliteal lymph node, there was an insignificant amount at the site of injection, even though many granulocytes and macrophages were present. When they injected dysentery and typhoid antigens in combination into the abdominal cavity, there were no antibodies in the isolated granulocytes and macrophages of the exudate of the peritoneum, while the supernatant fluid showed antibody titres nearly as high as those of the blood serum. They got similar results when they first injected animals intravenously with antibody, and, subsequently, with an unspecific irritant intraperitoneally. From these experiments they concluded that "macrophages and granulocytes do not synthesize agglutinins against dysentery and typhoid bacilli".

Ehrich and Harris (1946) attempted to outline the developments in local and lymphatic tissue between the time that the antigen is injected and the first appearance of antibodies. They made extracts of the regional lymph nodes adjacent to the injected tissue, and examined these and the composition of efferent lymph from the node. The antigen fell off quickly in the extracts and the lymph, and its disappearance was succeeded by the appearance of antibodies. Their idea is that after particulate antigenic material is injected, these particles are subjected to a physiologic process which results in the liberation of smaller soluble particles. The latter retain groupings which are immunologically characteristic of the original antigen. They are carried by the lymph stream to the regional lymph nodes where they come in contact with lymphoid tissue, thus stimulating the lymphocyte which is the antibody synthesizer.

The results of all these experiments still do not clearly indicate that phagocytosis and antibody formation are similar processes. It is quite plausible that the macrophages and granulocytes simply prepare the antigen, either digesting or dissolving it, and the products of the digested antigen induce the lymphocyte to produce antibodies. The antigens would then "train" lymphocytes to produce antibodies rather than to release the normal gamma-globulin present (Ehrich 1946).

The Plasma Cell and Antibody Production

It may also be that globulins in plasma cells, which are also present in the lymph nodes, can give rise to antibodies. Lymph nodes from which the amount of antibody present was determined also contain some plasma cells. The latter may have become included in the extracts made of the nodes by shedding their cytoplasm which was centrifuged in turn with the lymphocytes and remained in the extract throughout all the investigation. Hyperglobulinemia is usually associated with an increase in plasma cells in the bone marrow and elsewhere, whereas in lymphatic leukemia, the globulins are at normal levels (Ehrich 1946).

Bjorneboe, Gormsen, and Lundquist (1947) investigated the role of the plasma cells as antibody producers. They immunized rabbits with a mixture of eight pneumococcus types, and produced a massive plasma cell infiltration (90 per cent) and a slight lymphocyte infiltration (10 per cent) in the adipose tissue of the renal sinus. Extracts of the adipose tissue of the renal sinus, which was rich in plasma cells, contained a great amount of antibody-protein, essentially more than was found in the extracts of any other organ from these animals. The authors have, therefore, advanced the hypothesis that antibodies are produced by plasma cells. This work, however, is not conclusive enough to ascribe antibody production

entirely to the plasma cell.

FACTORS AFFECTING ANTIBODY PRODUCTION AND RESISTANCE TO INFECTION

Adrenal Cortical Control of Lymphoid Tissue

It has been shown by many investigators that the thymus has an inverse relationship with the amount of adrenal cortical secretion. Jaffé (1924) found that when rats were bilaterally adrenalectomized, they developed a secondary hyperplasia of the thymus resulting in an enlargement of the organ. Moon (1937) found that both male and female castrated rats which were given adrenocorticotropic hormone (A.C.T.H.) developed very atrophic thymi, and, in some cases, complete atrophy occurred. Ingle (1938) found that when normal rats were given cortin, a marked involution of the thymus occurred. He also hypophysectomized some animals, to half of which he gave A.C.T.H., and cortin along with the A.C.T.H. to the rest. The latter revealed a marked involution of the thymi, whereas in the former, the thymi were similar to those in the normal animals. In 1940, he observed the effects of two steroid compounds on the size and weight of the thymus. He adrenalectomized a number of rats, and separated them into three groups. To the first, he administered 2.0 mg. of 17-hydroxy-11-dehydro-corticosterone daily; to the second, he injected 2.0 mg. of 11-desoxycorticosterone acetate (D.O.C.A.) daily; and to the third, 10.0 mg. of D.O.C.A. daily. The first group revealed a still more marked involution of the thymus than did the bilaterally adrenalectomized animals. The second set of rats showed a gain in body weight, but no significant regression of the thymus, whereas the last set demonstrated a definite loss in the weight of the thymus, but the extent of the atrophy was less than that of the first group.

Up to this time, nothing was known about the effects on the rest of the lymphoid structures of the adrenal cortical secretions. Reinhardt

and Holmes (1940) in investigating this problem found that adrenalectomized rats did not show a decrease in the weight of the thymus and the lymph nodes, but were even heavier than the normal animals. Histological examination of these structures revealed an increase in the bulk of the organs, but no distortion in their appearance. Dougherty and White (1943) showed that injections of A.C.T.H. into normal animals produced the opposite effect. There was a decrease in the weight of the axillary, inguinal, and mesenteric nodes, and of the thymus, whereas the spleen showed no changes whatever. Simpson, Li, Reinhardt, and Evans (1943) demonstrated this same phenomenon. Dougherty and White (1944) found that a daily injection of A.C.T.H. caused a marked decrease in the lymphoid tissue mass. Within a few hours after the administration of the hormone, the lymphoid tissues became swollen and edematous, with degenerating lymphocytes in the edematous fluid of the medullary portions of the thymus and lymph nodes. Initially, then, there occurred an increase in the weight of the tissues, but the edema subsided after 15 hours, the fluid was lost, the lymphocytes became fewer in number, and the weight of the tissue was considerably less than that in the normal animals. When the involution finally occurred, a marked lymphopenia also occurred in both the lymphoid masses and the body fluids. Further evidence for this was given by Reinhardt and Li (1945) who found that A.C.T.H. depressed the lymphocyte count of the thoracic duct lymph. However, this did not seem plausible as one would expect an increase in blood lymphocytes when they decreased in number in the lymphoid tissues. What was the fate of these lymphocytes?

Histologic examination of the lymphoid tissues following the administration of a single injection of A.C.T.H., as early as one hour afterward, revealed that the lymphocytes began to degenerate (Dougherty

and White 1945). A great deal of debris accumulated which was phagocytosed gradually by the macrophages giving the organ a pitted appearance. The germinal centers exhibited the initial effect of lymphocyte degeneration, and this spread to the rest of the node. This occurred within the first nine hours after the injection, and was followed by a reparative phase wherein phagocytosis took place more rapidly and mitotic figures began to appear in the germ centers. The organs returned to their normal appearance, but they did not contain as many lymphocytes as were present normally. The lymphopenia was a result of the "dissolution" of lymphocytes, and this accounted for their disappearance from the circulation. The dissolution was characterized by three alterations: 1) an increased loss of cytoplasm due to the hormone administration, 2) a destruction of the nuclei by both karyolysis and karyorrhexis, and 3) a development of hyaline granules in the cytoplasm which was shed from the cells and found in the lymph. The changes which occurred to the lymphocytes were due to a physiologic action of the adrenal cortical hormones on them. The lymphocyte is then the target cell of these hormones, in normal amounts.

Dougherty and White (1944, 1945) also found that continued daily injections of A.C.T.H. caused, in addition to an absolute lymphopenia, an increase in hemoglobin and the number of erythrocytes, and the number of polymorphonuclear leucocytes. Murphy and Sturm (1944) in observing the effect of A.C.T.H. on transplanted leukemia in rats found that normal animals which were first injected with leukemic cells, and hours afterward received hormones, had a lower death rate than did the animals which did not receive the hormones in addition to the leukemic cells. There was a great deal of variability in the results, so that they were not too conclusive. This work, however, supplements the previous work of

other investigators indicating that there is hormonal control of the lymphoid tissue.

Adrenal Cortical Control of Serum Globulins

The loss of the lymphocytes from the tissues and the circulation led the investigators to determine the constituents of the cytoplasm shed in the dissolution. White and Dougherty (1944) studied the alteration of the blood serum proteins at various intervals following the administration of A.C.T.H. and adrenal cortical hormones, for the lymphocytes were obviously rich in protein since they had been described as the site of antibody formation. Three to six hours after a single dose of A.C.T.H. when maximum lymphopenia and lymphocyte dissolution existed, there was an increase in the level of the total serum proteins. Shortly after six hours, it returned to normal. The blood serum proteins remained high when continued doses of A.C.T.H. were administered. Electrophoretic examination of the serum proteins showed that both the beta- and gamma-globulin fractions were increased with no change in the amount of alpha-globulin, when A.C.T.H. was given. The total albumin concentration of the serum was lowered, but the authors find this of little significance. Adrenalectomy and adrenal cortical hormones resulted in a decrease of serum globulins. Lymphoid tissue is then the source of these serum globulins and A.C.T.H. controls the rate of their release.

Dougherty, Chase, and White (1944) revealed through their experiments the presence of antibody globulin in the lymphocyte. They made extracts of the lymphocytes of non-immunized and immunized animals. The former did not contain any labeled globulins (antibodies). The latter, however, did, as did the serum of the immunized animal. The antibody titre of the serum was lower than that of the lymphocytes, even though

it contained three to four times as much nitrogen as did the lymphocyte extracts. Dougherty, White, and Chase (1945) demonstrated by similar experiments that malignant lymphocytes also contain antibodies.

Adrenal Cortical Control of Antibody Production

Dougherty et al. (1945) conducted two types of experiments. In the first one, they immunized rabbits to sheep erythrocytes, and when sufficient antibody had appeared, they stopped the injections and allowed the animals to remain in the laboratory for about three months, until no circulating antibody could be detected in their blood. In one group of animals, they administered a single injection of cortical steroids in oil; in a second group, a single dose of A.C.T.H.; in the third, a single injection of adrenal cortical extract; and in the fourth, a single injection of the original antigen, the sheep erythrocytes. The group which received the sheep cells gave the lowest titre of antibodies, whereas the one which received the cortical steroids in oil yielded the highest titre. A.C.T.H. was half as effective as the cortical hormones, and the aqueous adrenal extract was one-fifth as effective as the cortical hormones in oil.

In the second experiment, they immunized mice with sheep erythrocytes until the antibody titre of the blood was 1:640. Immunization was stopped, and the animals remained in the laboratory until they exhibited no antibody titre. Lymphocyte extracts of these animals exhibited a significant quantity of antibody. The subcutaneous injection of an aqueous adrenal cortical extract in these animals produced a release of antibodies from the lymphocytes into the serum in three hours. This extract was also capable of restoring the ability of adrenalectomized animals to release their antibodies. Adrenal cortical steroids in oil

were also effective in releasing the antibody, whereas D.O.C.A. was ineffective. Two other agents, benzene and potassium arsenite, produced effects on lymphoid tissue physiologically identical with those seen with the administration of A.C.T.H. and adrenal cortical extracts. This did not occur in adrenalectomized animals, however (White and Dougherty 1946).

Chase, White, and Dougherty (1946) immunized various animals with sheep erythrocytes, staphylococcus, horse serum, and egg albumin. When A.C.T.H. and adrenal cortical hormones were injected in addition to the various antigens, the antibody titre of the blood was twice as great. They suggested that the enhancement of the antibody titre was due to an increased rate of release of antibody from lymphocytes effected by augmented amounts of A.C.T.H.

It is concluded, therefore, from these researches, that the adrenal cortex, and, in turn, the pituitary which has control of the adrenal cortical secretions, have a specific effect on lymphoid tissue histology, on the number of blood lymphocytes present in the blood and lymphoid tissue, on the level of certain serum proteins and consequently of the beta- and gamma-globulins, and on the rate of release of antibody from the lymphocytes in immunized animals. In adrenalectomized animals, the pituitary hormone has no effect. These animals have significant quantities of antibody in their lymphocytes, but are unable to release them until adrenal cortical hormones are administered.

Protein Deficiency and Antibody Production

Since normal globulin results from the utilization of dietary protein, food protein must play an important role in the production of antibodies. Cannon (1942) and Cannon, Chase, and Wissler (1943) experimented with the effects of protein reserves and plasmaphoresis on antibody. They made young rabbits hypoproteinic by a low protein diet.

Plasmaphoresis was induced by removing 25-50 cc. of blood from the animals, either daily or on alternate days until 150-200 cc. were withdrawn. In each case, the blood was mixed with sodium-citrated isotonic saline solution and centrifuged in an angle centrifuge for 10 minutes at 3,000 r.p.m. The plasma was then decanted, and the packed cells were resuspended in Locke's solution containing no glucose or calcium equal in volume to the blood originally withdrawn. This was injected into the marginal vein of the rabbit's ear. Protein depletion was determined at various intervals, before and after the diet was used, and before the immunization of the animals, by the estimation of serum protein levels of the body. They found that the hypoproteinic animals exhibited a definitely lowered capacity to produce agglutinins than did normal animals of similar age fed on a well balanced diet. They found that adult rabbits made hypoproteinic by a low protein diet or by a low protein diet supplemented by plasmaphoresis also exhibited a decreased capacity to produce agglutinins as compared with animals of similar age on an adequate diet.

Cannon (1944) published a report wherein he compared protein metabolism and resistance to infection. He stated that blood proteins were decreased either by an excessive demand of the body upon them for energy, or by a decreased intake of dietary amino acids. Progressive lowering of plasma protein concentration ultimately caused nutritional edema, intercurrent sepsis, and eventually death. It was this protein loss which caused the basic foundation of immunity; i.e., the formation of antibodies, to be upset. The state of "dynamic equilibrium" in the body wherein tissue reserves of protein serve as a medium of nitrogen exchange and are in equilibrium with the plasma proteins is unbalanced. The production of normal globulin and antibody globulin are similar

processes, so that sufficient intake of protein constituents is necessary to keep both of them at normal levels. Cannon et al. (1945) showed how the loss of protein reserves made them less able to respond to infections. The depletion of the protein reserves also caused bone marrow cellular depletion which resulted in leukopenia and terminal sepsis. The reserve of leukocytes on hand at this time were unable to furnish an adequate number of phagocytic cells during a developing infection. Cannon therefore maintains that acquired immunity and natural resistance function more effectively when the nitrogenous nutriment is always present in the body to maintain the mechanism of antibody production and phagocytic cell formation in the bone marrow.

Wissler et al. (1946, 1946) observed the effects on antibody production when protein depleted rats were fed diets with high quantities of proteins. They found that the antibody-producing mechanism was quickly restored by feeding the animals adequate and well balanced quantities of amino acids coming from enzymatic protein hydrolysates, food protein, or mixtures of seven or more amino acids.

Biotin Deficiency and Resistance to Infection

Biotin deficiency in rats effected by feeding them raw fresh egg-white resulted in their diminished resistance to Salmonella typhimurium (Kligler, Guggenheim, and Herrnheiser 1946). Mice kept on a biotin deficient diet showed far greater susceptibility to aspontaneous Salmonella infection than did control mice from the same litter and stock which were fed vitamins A and E in addition to boiled fresh egg-white. Biotin deficiency had been shown to cause an early thymic involution with excessive connective and adipose tissue infiltrating the thymus (Shaw and Phillips 1942). It is probably this involution of the thymus and of other lymphoid masses which caused a decreased resistance to

infection by the Salmonella organisms.

Pyridoxine Deficiency and the Antibody Response

Pyridoxine deficiency in rats was seen to cause not only thymic involution, but also lymphoid tissue atrophy (Stoerk 1946). The thymi and lymph nodes of the deficient animals exhibited a marked reduction as well as did the circulating lymphocytes. Immunization was begun on animals when lymphoid tissue atrophy was advanced, and resulted in little or no circulating antibody (Stoerk and Eisen 1946). The investigators did not have sufficient data, however, to decide definitely that suppression of serum antibodies was a consequency of lymphoid atrophy. Further investigations by Stoerk, Eisen, and John (1947) showed a striking impairment of the antibody response to sheep erythrocytes in pyridoxine deficient rats. Deficiencies in three other vitamin B factors which are required by the rat, in addition to a low protein feeding, failed to influence the antibody response, even though the body weight decrease was comparable to that in the pyridoxine deficiency. The thymus and lymphoid tissues were also strikingly atrophied in the experimental animals.. Thiamine deficiency caused thymic involution but less pronounced effects on the other lymphoid structures. There is an inconsistency here, as the thiamine deficient animals had serum protein levels as high as those of the controls, even though they exhibited marked lymphoid tissue atrophy. Experiments are still going on to try to obtain more evidence to clarify these contradictory results.

SUMMARY

The mechanism of resistance to infection has been a topic of investigation since the time of Metchnikoff who introduced the "cellular" theory of immunity. Since this was not adequate to explain fully the phenomenon of immunity, the presentation of Ehrlich's "side-chain" theory was eagerly accepted by the investigators of that day, and it resulted in a long line of experiments on the specific antitoxic factors which were elaborated when toxins were introduced into the animal body. Since these elements (antibodies) were found in the blood stream following phagocytosis of the toxin (antigen), the investigators believed that they were formed by phagocytosing cells of the reticulo-endothelial system. Many experiments were performed with these cells in an attempt to find out whether or not they were the actual site of antibody formation, and, consequently, the main cells instrumental in counteracting infection.

Extirpation and damage to the various organs of the reticulo-endothelial system gave some evidence that the antigen was removed from the circulation shortly after it was introduced (Luckhardt and Becht 1911). It was found that the spleen was concerned in removing the antigen from the circulation, but it was not known whether it produced the antibodies. Topley (1930) elaborated this work and added that the splenic cells did not store the antigen only, but also produced the antibodies. Damage to the system by X-rays, benzene, and mustard gas showed that the reticulo-endothelial system was instrumental to a great extent in producing antibodies. These results, however, are not very conclusive as it is impossible to know whether any other damage has taken place to the other organs along with those of the reticulo-endothelial system.

Blockade was another method used in this study. It was effected by large intravenous doses of India ink or colloidal iron so that the cells of the system would become active phagocytosing these particles, and would not be able to deal with the antigen when it was introduced later. The variability of the results here also indicates that the method of investigation was not adequate in that it could not be definitely known whether or not the entire system had been blockaded.

Other experiments with the use of tissue cultures of the cells of the reticulo-endothelial system have also given a variety of results, so that no definite conclusions can be drawn from them. The most successful method of study was the determination of the amount of antibody contained in the various organs of the immunized animals. In most cases, the investigators located the antigen in the various organs and assumed the same amount of antibody was also present there. Sabin (1939) was able to show by the use of a red dye which cells phagocytosed the antigen and also presented evidence that these cells played an important part in the production of antibodies. However, since then a great deal of more conclusive work has been done ascribing the site of antibody production to lymphoid tissue and especially to the lymphocyte.

McMaster and Hudack (1935) demonstrated that antibodies could be produced in the lymph nodes, and that either the reticulo-endothelial cells or the lymphocytes could be responsible. Ehrich and Harris (1942) after analyzing the composition of the afferent and efferent lymph nodes during immunization, and observing the histological changes in the lymph nodes at this time, found that a lymphocytic response preceded and followed antibody production. Harris et al. (1945) continued their work. They experimented with the lymphocytes in the efferent lymph of immunized animals and found that the lymphocyte was instrumental in the

production of antibodies. This work was substantiated by the results of Dougherty et al. (1944) who also demonstrated that the antibodies were concentrated in the lymphocytes. Ehrich et al. (1946) ruled out the possibility that the cells of the reticulo-endothelial system played any part whatever in the production of antibodies, as they observed that the cells were entirely inactive during all the experiments.

It was shown that an inverse relationship existed not only between the thymus and the amount of adrenal cortical secretions present in an animal, but also between the latter and all the lymphoid tissue (Jaffe 1924, Moon 1937, Ingle 1938, 1940, Reinhardt and Holmes 1940, and Dougherty and White 1943). Dougherty and White showed that a "dissolution" of lymphocytes occurred when adrenocorticotropic hormone of the pituitary and adrenal cortical steroids were administered, and concomitantly there resulted an increase in the levels of the beta- and gamma-globulins of the serum, and an absolute lymphopenia of the lymphoid tissue and the entire circulation. In the immunized animal, there was an increase in the amount of antibody globulin in the serum (Dougherty et al. 1944-46). The dissolution of the lymphocytes was characterized by the shedding of their cytoplasm which was released to the lymph and the rest of the circulation. Since the lymphocyte was shown to contain four protein constituents, two of which were similar to beta- and gamma-globulins of the serum, it seemed quite evident that the shedding of the cytoplasm of the lymphocytes upon the administration of A.C.T.H. would contribute a large amount of lymphocyte globulin to the system. Dougherty et al. (1944) demonstrated that antibodies were present in the lymphocyte so that a release of these globulins into the serum produced a rise in the circulating antibody.

11-Desoxycorticosterone acetate was shown to be ineffective in

producing lymphocyte dissolution and, consequently, a release of antibody into the circulating blood, whereas the cortical steroids controlled the rate of lymphocyte dissolution (White and Dougherty 1944, and Dougherty and White 1945). Acute lymphopenia was evidenced in the normal animal by a release of globulin from the lymphocyte, and in immunized animals by the release of antibody when A.C.T.H. and adrenal cortical steroids were administered to intact animals.

The possibility that lymphoid tissue was important in protein metabolism arose when lymphocytes were shown to be a source of serum globulins. Since the lymphocytes are distributed all over the body, and they readily release their protein constituents upon the administration of A.C.T.H., it is possible that they store readily available proteins; namely, globulins. This protein is under direct hormonal control as it can only be released in the presence of the adrenal gland. Cannon et al. have demonstrated that a low protein diet affects the antibody-producing mechanism. The "dynamic equilibrium" which exists between the plasma and tissue proteins is upset in hypoproteinemia, so that the production of normal and antibody globulin which is in similar equilibrium is also upset. The hormonal control of the plasma proteins can be possible only when sufficient protein reserves are present. When an imbalance occurs, the mechanism for resistance to infection is not able to function correctly. Cannon et al. (1945) demonstrated this latter phenomenon. Their experiments showed that a lowered intake of protein nitrogen by an animal caused a depletion of its protein reserves, a decrease in the amount of lymphoid tissue present in the animal, and, consequently, a lowered resistance of the animal to infection. Wissler et al.(1946) showed that when hypoproteinic animals were fed high quality proteins, their ability to counteract infection was restored.

Plasma cells whichare rich in globulin and are active in the formation of globulins may also be the site of antibody formation. Bjorneboe et al. (1947) have demonstrated that the plasma cells are instrumental in producing antibodies, but more investigation is necessary.

Vitamin deficiencies such as acute biotin and pyridoxine deficiency has been shown to cause a reduction in the masses of lymphoid tissue in the body, and also a lowered resistance to infection. Conflicting results have been obtained from the pyridoxine deficiency experiments, so that no definite conclusions can be drawn at this time, as to their effects on lymphoid tissue and on antibody formation.

Although a great deal of evidence has been presented ascribing the site of formation to the lymphocyte and to lymphoid tissue as a whole, no definite conclusion can be made at this time. Polymorphonuclear leucocytes, macrophages, and plasma cells may also be instrumental in their production. Further experimentation is necessary.

ABSTRACT

This review presents evidence for the nature and mechanism of the formation of specific factors (antibodies) which are very important in counteracting infection.

In the introduction, a brief review is included beginning with Pasteur's early ideas about immunity, Metchnikoff's "cellular" theory, Ehrlich's "side-chain" theory, and all the recent investigations which have shown that antibodies are produced in lymphoid tissue and especially in the lymphocyte. A complete anatomical and histological description of lymphoid tissue and the lymphocyte is also given.

Earliest theories stated that phagocytosis by macrophages was the main mechanism in counteracting infection. Then it was found that specific antibodies were produced which were more instrumental in producing resistance to infection. However, investigators have shown that both phagocytosis and antibody formation are necessary. The nature of the cells which did the phagocytosing had been known for a long time, but this was not true for the source of the antibodies. The reticulo-endothelial system which is composed of phagocytosing elements was thought, at first, to be the site of antibody formation also. Evidence for this was based on experiments wherein extirpation and damage of the organs of that system, blockade and tissue cultures, and measurement of the antibody content of the various reticulo-endothelial organs in immunized animal were carried on. It was only the latter study, however, which gave any conclusive results whatever.

In 1937, a series of experiments were begun which finally led to the conclusion that the lymphoid tissue and especially the lymphocyte were instrumental in producing antibodies. Even more recent investigations seem to ascribe this function to the plasma cell. However, more experi-

mentation is necessary along these lines.

Adrenal cortical hormones and the adrenotropic hormone of the pituitary have been shown to possess control of the amount of lymphoid tissue present in the body, the rate of release of serum globulins from the lymphocytes, and the amount of circulating antibody in an immunized animal. The effects of protein and vitamin deficiency in connection with lymphoid tissue, antibody production, and consequently resistance to infection are also discussed.

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